## **SHORT COMMUNICATION**

## The rate of visitation by *Amazilia fimbriata* (Apodiformes: Trochilidae) influences seed production in *Tillandsia stricta* (Bromeliaceae)

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ABSTRACT. Legitimate flowers visitors pollinate the flower during the visit and thus influence the production of fruits and seeds. We tested whether the visitation rate of potential pollinators is associated with the amount of seeds per fruit produced by the self-compatible bromeliad *Tillandsia stricta* (Bromeliaceae). We determined whether hummingbirds are legitimate visitors by testing for a correlation between visits and pollination (seed production) at the Guapiaçú Ecological Reserve (Reserva Ecológica de Guapiaçú), state of Rio de Janeiro. We tested 30 flowers, five of which were also monitored to test the possibility of spontaneous self-pollination. The remaining 25 flowers were exposed to floral visitors. Twenty-two flowers formed fruits and seeds, from which three formed seeds without floral visits. The humming-bird *Amazilia fimbriata* (Gmelin, 1788) was the only legitimate visitor. The average number ( $\pm$  standard deviation) of seeds was 27 units ( $\pm$ 15) per fruit. The floral visitation rate by *A. fimbriata* was 6.6 ( $\pm$ 3.4) visits/per flower. The number of floral visits and the amount of seed produced were positively correlated ( $r^2 = 0.58$ , p < 0.01). Thus, *A. fimbriata* is a legitimate floral visitor of *T. stricta*, and influences seed production per fruit in this bromeliad.

KEY WORDS. Atlantic forest, hummingbird, pollination, self-compatibility.

Bromeliaceae includes 58 genera, of which *Tillandsia* Linnaeus is one of the richest, with about 600 epiphytic or rupicolous species (Luther 2008). Species in this family are pollinated mostly by vertebrates, and hummingbirds are their most frequent visitors (Zanella et al. 2012). The effects of plant-pollinator interaction on plant reproduction, however, are still poorly understood (Antonelli & Sanmartín 2011, Kamke et al. 2011, Zanella et al. 2012). Species in this family have two main breeding systems, and self-compatibility (SC) is more common than self-incompatibility (SI). SC may be a pre-zygotic reproductive isolation mechanism in Bromeliaceae populations that are subjected to interspecific pollen flow (Wendt et al. 2008, Matallana et al. 2010).

In SI species, an increase in the visitation rate of pollinators is expected to be positively correlated with fecundity, since pollinators increase the number of pollen grains transported (Longo & Fischer 2006) only if pollen is limited (Hargreaves et al. 2009). This relationship occurs because cross-pollination is necessary for fertilization (Takayama & Isogai 2005). However, even in SC bromeliad species, floral visits may increase pollen transfer between floral structures and positively influence fecundity (Siqueira Filho & Machado 2001).

Tillandsia stricta Sol is an epiphytic, ornithophilous species with a SC reproductive system (MATALLANA et al 2010). It is

widely distributed in Brazil (Pontes & Agra 2006, Cogliatti-Carvalho et al. 2008), occurring in the Atlantic forest and its associated ecossistems (Cogliatti-Carvalho et al. 2001, Bonnet & Queiroz 2006, Machado & Semir 2006). This species receives floral visits from different species of hummingbirds (Apodiformes: Trochilidae) and from the bird Bananaquit, *Coereba flaveola* (Linnaeus, 1758) (Passeriformes: Thraupidae), in addition to different insect species (Sazima et al. 1996, Alves et al. 2000, Machado & Semir 2006). In the present study we tested the hypothesis that flower visits by potential pollinators of *T. stricta* are positively correlated with the number of seeds produced per fruit.

The study took place in the Atlantic Rainforest within the Guapiaçú Ecological Reserve (22°24′S, 42°44′W, 7,000 ha) near the city of Cachoeiras de Macacu, state of Rio de Janeiro. We set up the experiment and observed flower visitors during six days in January 2013, and returned to collect fruits in March 2013.

We monitored 30 flowers from six individuals of *T. stricta*, including five flowers used to test for spontaneous self-pollination. The remaining 25 flowers were bagged with tulle fabric to prevent access of flower visitors.

It was not possible to limit our survey to one flower per individual because the plants were difficult to assess. We used one to three flowers per individual per day to make the observations on flower visitors. As we needed up to three days to conclude de observations of each flower, several flowers per individual were necessary to achieve a minimum sample size (about 12 flowers observed per day). The distance between the individuals sampled was about 20 m, and these individuals were in similar environmental conditions: two meters up from ground level in riparian vegetation. Each inflorescence had one to five flowers in anthesis per day.

During anthesis, we uncovered the flowers and exposed them to flower visitors between 6:00 a.m. and 6:00 p.m. After that we bagged them again. Observations were made for 12 consecutive hours on each flower sampled. We applied a thin layer of the non-toxic resin Tanglefoot® at the base of each flower stalk to block access by non-winged insects (Del-Claro et al. 1996). This process was repeated until the flower entered into senescence (three days). Stigma viability was determined using hydrogen peroxide (Kearns & Inouye 1993) on unmonitored flowers of the same individuals. During focal observations we recorded floral visitor species and visit legitimacy (INOUYE 1980). After approximately 60 days, we collected fruits from the monitored flowers and determined the amount of seeds in each fruit. The flower stalks and resin were removed after the experiment. We tested the influence of the frequency of hummingbird floral visits on the number of seeds using a simple linear regression.

During the six-days of observation, flowers were visited only by the hummingbird Amazilia fimbriata (Gmelin, 1788). The flowers opened at dawn and closed at dusk. Each flower lasted for two to three days before entering into senescence. Among the five flowers monitored for spontaneous self-pollination, two formed fruits without the help of floral visitors. Of the other 25 monitored flowers, 22 formed fruits during the period considered (60 days), including three flowers that formed fruits without receiving floral visits. The remaining 19 flowers received an average ( $\pm$  standard deviation) of 6.6 ( $\pm$ 3.4) visits throughout their duration. Each fruit had an average of 27 ( $\pm$ 15) seeds. Fruits formed by spontaneous self-pollination produced less seeds with an average of 9.3 ( $\pm$ 2.3) seeds per fruit. The amount of floral visits and the number of seeds produced per fruit were positively correlated ( $r^2$  = 0.58, F = 24, df = 1.17, p < 0.01) (Fig. 1).

Flowers of T. stricta are typically receptive for three days, and may only be receptive the day after anthesis, even though pollen can be released soon after anthesis (Machado & Semir 2006). The anthers of the T. stricta flowers monitored by us released pollen and their stigmas were receptive shortly after anthesis. Increased seed production as a consequence of an increase in the number of visits by A. fimbriata shows that this bird is a legitimate pollinator. Similarly, hummingbird visitation resulted in greater seed production by  $Canistrum\ aurantiacum\ E$ . Morren (Bromeliaceae) than spontaneous self-pollination or manual pollination (Siqueira Filho & Machado 2001).

The fact that the only floral visitor of *T. stricta* in our study was a hummingbird contrasts with the results of two studies on species of the genus: ALVES et al. (2000) found that

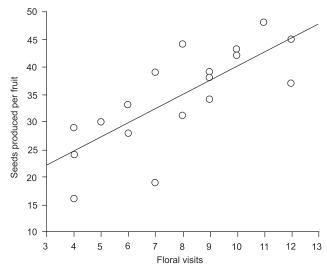


Figure 1. Linear regression between the rate of visitation of *Amazilia fimbriata* and the prodution of *Tillandsia stricta* seeds.

insects were the most frequent visitors of this and other *Tillandsia* species (Varassim & Sazima 2000), and Machado & Semir (2006) found more than one hummingbird species visiting *Tillandsia* flowers. Our results indicate that increased foraging activity by one hummingbird species can stimulate seed production in *T. stricta*. We believe that an increased number of pollinator species, leading to higher visitation rate, could help to increase the fertility of this bromeliad, but this hypothesis needs to be tested

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